Protocol: Chemotaxis on plate MB

Aim: Observing bacterial chemotaxis on plate or carrier glass with coverslip.

Materials:

- Plate or carrier glass with coverslip..
- inverted microbiology microscope (can get to X100).
- Repellent/attractant at different concertation:
    - Attractant: Asp 1mM (final concentration)
    - Repellent: Benzoate 50*10^-6 M, Nitrite/Nitrate 10^-5 – 1 M (final concentration)
    - Control: Motility buffer
- 7Bacterial strains (O.D 0.6-0.9)
    - Positive control – MG, deltaZras
    - Negative control – deltaFliM
    - Tested: PctA(a),Tar1(a/r),Tar2(a/r),NarX(r)
- BSA 2%
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- Note: Benzoate is most effective at pH 5.5
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Protocol:

First Day:

1. O/N at 10 degrees in TB medium
2. Prepare dilutions of attractants and repellents

Second Day:

1. In the morning change temperature to 30 degrees till O.D 0.6-0.9
2. Change the media to motility buffer
3. Wash carrier glass with BSA and then with water
4. Pipette 8 µl bacterial solution on the plate or carrier glass.
5. Place the plate inside the microscope.
6.
7. Set it to X50-X100.
8. Pipette 2 µl of the repellent/attractant. While using cover slip pipette the material on the cover slip and place on the glass or stir.
9. Look at the results through the microscope while recording it from the moment of contact with the bacteria. Film around 2 minutes or adaptation.
10. Continue recording until adaptation.
11. Repeat all steps around 10 times.
12.
13. write the results.

Notes:

1) Wash with BSA so the bacteria won't stick to the glass
2) The final volume should not exceed 10 µl
3) using an injector instead of a pipettor is recommended
4) Good control can be a strain that does not adapt (does not stop chemotaxis due to adapting to concentration – Oshri's lab has this stra